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NOISE ANALYSIS IN STUDIES OF PROTEIN DYNAMICS AND MOLECULAR TRANSPORT

SERGEY M. BEZRUKOV
Laboratory of Physical and Structural Biology, NICHD, National Institutes of Health, Bethesda, MD 20892-0924, USA
bezrukov@helix.nih.gov

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Understanding the role of noise at cellular and higher hierarchical levels depends on our knowledge of the physical mechanisms of its generation. Conversely, noise is a rich source of information about these mechanisms. Using channel-forming protein molecules reconstituted into artificial 5-nm-thick insulating lipid films, it is possible to investigate noise in single-molecule experiments and to relate its origins to protein function. Recent progress in this field is reviewed with an emphasis on how this experimental technique can be used to study low-frequency protein dynamics, including not only reversible ionization of sites on the channel-forming protein molecule, but also molecular mechanisms of 1/f-noise generation. Several new applications of the single-molecule noise analysis to membrane transport problems are also addressed. Among those is a study on antibiotic translocation across bacterial walls. High-resolution recording of ionic current through the single channel, formed by the general bacterial porin, OmpF, enables us to resolve single-molecule events of antibiotic translocation.

Keywords: Ion channels; single molecules; fluctuations; bacterial porins.

1. Introduction

The last decade saw an explosion in biologically motivated noise research. The 2002 conference 'Unsolved Problems of Noise in Physics, Biology, and High Technology' held at the National Institutes of Health campus in Bethesda demonstrated significant progress in our understanding of noise at the many levels of biological organization, from single protein molecules, to neurons, to the human brain [1]. One of the leading themes of this research is the constructive role of noise in biology, the theme whose recent successful elaboration would have been impossible without landmark contributions of its ardent proponent and pioneer, Professor Frank Moss, and his colleagues [2–6].

The comprehensive picture of the constructive role of noise necessarily includes quantitative understanding of the physical mechanisms of noise generation. It is widely accepted that for neuronal systems one of the most important noise sources is related to the probabilistic gating of membrane ion channels [7, 8]. In retrospect, noise analysis of

membrane ion currents played a crucial role in verifying the existence of protein ion channels — key membrane-bound structures that allow cells to sense changes in the chemical composition of the media, communicate with each other, and maintain homeostasis. The original measurements revealed channel conformational kinetics and conductance and, therefore, the channel density in the cell membrane [9–12]. Today, with the development of the single-channel reconstitution technique, noise and fluctuation analysis is opening new possibilities in studies of molecular dynamics.

Advances in studies of the dynamics of complex biological molecules [13, 14] rely on the development of new experimental techniques and the continuous refinement of existing methods. Optical studies of single protein molecules [15] are among most powerful approaches. Observations of enzymatic activity at the single-molecule level reveal rich behavior and conformational memory in enzyme operation. This is seen as a non-Markovian fluorescence signal with non-exponential state transition probabilities [16]. Noise analysis of the ionic current flowing through a single ion channel [17] — a nanocontact formed by a single protein molecule — allows one to address similar questions using resolution that is often higher than in single-molecule fluorescence experiments.

Protein channels are 'soft' structures in the sense that their biological function depends on protein ability to respond to moderate changes in its immediate environment. Channels respond to changes in applied voltage, proton concentration, temperature, applied mechanical tension, etc. by changing their conformation. To be able to respond, the difference in free energies of functionally important conformational states of the protein should be comparable to kT, where k is the Boltzman constant and T is the absolute temperature. The activation barrier for such a conformational transition should also be small to provide for a reasonable response rate.

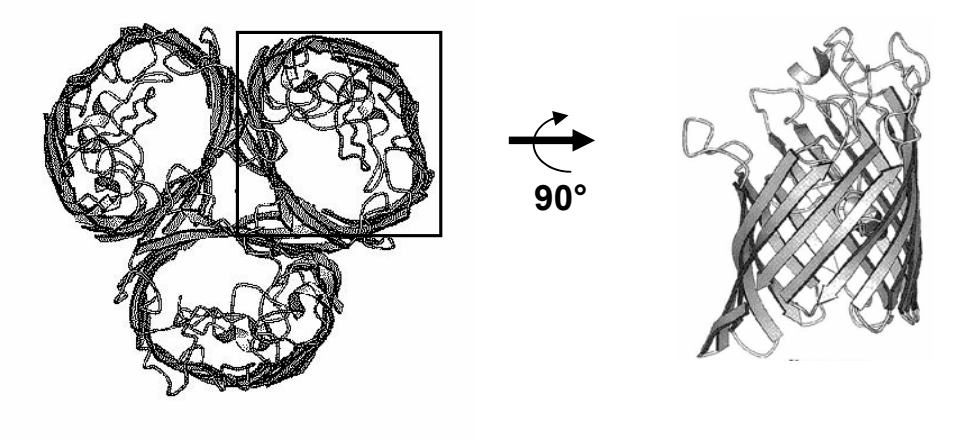

Fig. 1. High resolution X-ray structure of the channel formed by general bacterial porin, OmpF, viewed from out of the plane (left) and in the plane of the lipid film (right, only one monomer shown). The protein trimer has an overall diameter of about 10 nm. The main elements of the structure are three rigid β-barrels with several flexible loops folding into them (after [18]).

Even a brief glance at the channel architecture suggests a multitude of different physical processes that may contribute to the overall noise in the ionic current flowing

through the channel. Figure 1 shows the structure of the main bacterial protein channel, OmpF, that makes the outer membrane of bacteria permeable for metabolites and other small solutes. OmpF is a trimer with three water-filled pores. In each monomer, sixteen β-strands span the outer membrane and form a barrel with short turns at one side of the membrane and large loops at the other. Several of the loops are packed inside the channel. One of the loops folds into the barrel to form a constriction zone at half the height of the channel.

Table 1 lists noise sources in their approximate hierarchical order. It combines the well-known results from different laboratories [9–12] with those obtained in our studies. The spectral power ranges in the table give typical orders of magnitude only; they are not intended to set any limitations or serve as definitions. Below we discuss several noise generation mechanisms in more detail.

Table 1. Hierarchy of noise in mesoscopic ion channels.

Noise Source	**Spectral Type**	**Amplitude Range*, A^2/Hz**
Conformational Dynamics	Lorentzian, 1/f	$10^{-21} – 10^{-29}$
Blocker Binding	Lorentzian	$10^{-22} – 10^{-27}$
Residue Ionization	Lorentzian	$10^{-23} – 10^{-29}$
Neutral Solute Transport	Diffusional, Lorentzian	$10^{-24} – 10^{-29}$
Lipid Ionization	Diffusional, Lorentzian	$10^{-29} – 10^{-30}$
Ion Shot Noise	White	$10^{-29} – 10^{-30}$
Johnson Noise	White	$10^{-29} – 10^{-30}$

*Given for a single 'typical' mesoscopic channel of 0.1 to 1.0 nS conductance at 100 mV transmembrane voltage in the low-frequency decade of the sonic range.

2. Reversible Residue Ionization

Even for large protein channels, the water-filled pores are only about one nanometer in radius. This is the scale of the Debye screening length in water solutions with salt concentrations in the physiological range. Therefore, the charge state of the protein side chains that line the surface of the water-filled pore is crucial for channel ionic conductance. Strong electric fields produced by the side chain charges perturb the local distribution of ions and their mobility in the vicinity of the charge.

The charge state of the protein side chains depends on the proton concentration in the bulk solution, $[H^+]$. Bulk protons are in a dynamical equilibrium with side chain protons. For example, the average charge of the water-exposed carboxyl groups of glutamic and aspartic acids can be close to one half of the electron charge for a bulk proton concentration in the vicinity of 10 μM. Bulk protons hop on and off these side chains, thus modulating channel conductance and producing measurable fluctuations in the ionic currents through open channels [19–21].

Each monomer of the OmpF channel (Fig. 1) contains several dozen ionizable side chains, so it is natural to anticipate that their reversible ionization would be seen in its current noise. Indeed, this is the case. The results of noise measurements [22] are pre-

sented in Fig. 2. As expected, the open channel noise varies with the bulk proton concentration (pH = -*log*[H^+]).

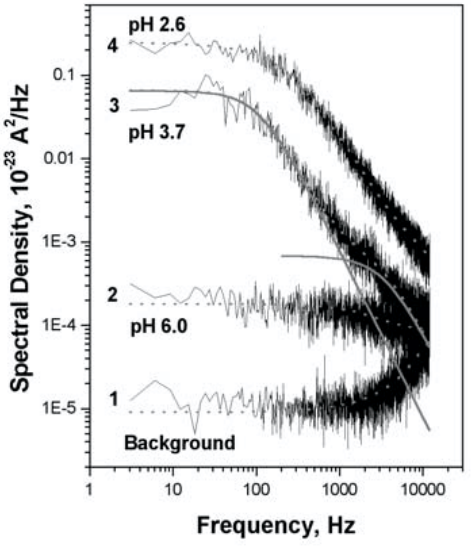

Fig. 2. Power spectral density of noise in ionic current through a single open channel of OmpF (Fig. 1) at different proton concentrations reveals dynamics of reversible ionization of the sites on the channel-forming molecule. The applied voltage was 100 mV; the OmpF was reconstituted into lipid films formed by opposition of two diphytanoyl phosphatidylcholine monolayers in aqueous solutions of 1 M potassium chloride. The background spectrum is measured at zero applied voltage [22]. The dotted lines are drawn to guide the eye; the solid lines represent an attempt of decomposition of the pH 3.7 spectrum into slow and fast Lorentzians.

The dominant low-frequency components are Lorentzians that allow us to determine the main characteristics of the residue ionization processes, such as the 'on' and 'off' rate constants and the influence of a single ionization event on channel conductance [19, 22]. This knowledge, taken together with the high-resolution structural information (Fig. 1), is very helpful in understanding electrostatics of the protein's nanoscale world.

3. 1/f Noise at the Single-Molecule Level

Our approach allows us to study 1/f noise at the level of a single molecule. Recently we have shown [23] that 1/f noise measured in the currents through multi-channel membranes [24] can be traced down to the step-wise conductance changes in a single 'fluctuator'. These step-wise changes have broad distributions of amplitudes and durations and reflect the complexity of protein structural organization and dynamics [13–16].

Figure 3 surveys the results of this study for the Maltoporin channel, whose structure is similar to the OmpF channel structure shown in Fig. 1, although its conductance is much smaller. The Maltoporin channel also exhibits 1/f noise that is absent in the case of OmpF (Fig. 2). Using high-resolution ionic current recording we have shown that the nanocontact formed by Maltoporin stochastically switches between sub-states of slightly differing conductance. The dynamics of these transitions are only weakly voltage-

dependent, which suggests their equilibrium nature. We have found that the current through the contact is virtually 1/f-noise-free in any of the sub-states and concluded that the 1/f noise is generated by random transitions between them.

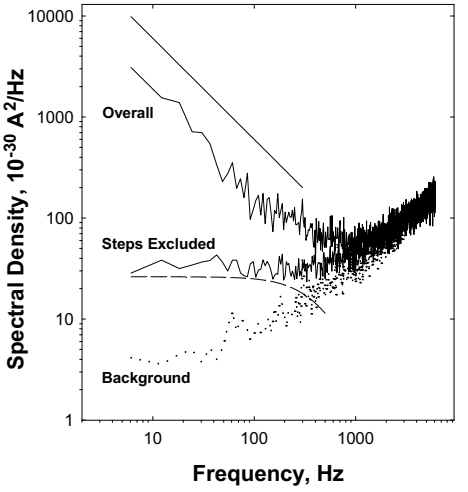

Fig. 3. Noise of a single open Maltoporin channel changes from 1/f-like behavior (upper curve) to 'white' one (middle curve) when step-wise transitions in the channel current are excluded [23]. The straight solid line shows the 1/f slope. The dashed line is a Lorentzian fit to the spectrum represented by the middle curve with the background subtracted. Lipid, salt solution, and applied voltage are as in Fig. 2; solution pH is 7.4.

Excision of the step-wise changes in the channel current transformed its low-frequency 1/f-like noise to noise with a flat spectrum. Importantly, very similar results could also be obtained using a selection of transition-free fragments of 1 s and longer by visual examination of current recordings. After subtracting the background, this noise component could be roughly approximated by a Lorentzian with a cut-off frequency of about 300 Hz. The intensity of its frequency-independent part is about $2.8 \cdot 10^{-29}$ A^2/Hz. This is still several times higher than the corresponding values for shot-noise, $2e\langle i \rangle = 5.8 \cdot 10^{-30}$ A^2/Hz (where $\langle i \rangle = 1.8 \cdot 10^{-11}$ A is the current through the single channel and e is elementary charge), or Johnson noise, $4kTg = 2.9 \cdot 10^{-30}$ A^2/Hz (where $g = 1.8 \cdot 10^{-10}$ S is the channel conductance) expected in this case. The origin of this excess noise is not understood presently. It may be related to the still unresolved open channel conformational fluctuations or to the reversible recharging of ionizable sites in the channel-forming molecule that was discussed above. In any case, the 1/f component observed in the analysis of overall recordings is absent here to a high accuracy. The upper estimate for the Hooge parameter obtained from the data in Fig. 3 is $\alpha \leq 3 \cdot 10^{-7}$.

Qualitatively similar conclusions were recently reached in a study of ion current noise through nanofabricated synthetic pores [25]. Noise with the spectral distribution of $1/f^{\alpha}$ was attributed to the motions of channel wall constituents.

4. Channel-Facilitated Molecular Transport

Fluctuations in the channel's ionic current may be induced by the particles passing through the channel. The idea is that nanometer-scale particles going through a structure of the type shown in Fig. 1 create transient interruptions in the ionic conductance [17, 26-28]. These transients generate measurable excess noise and, sometimes, can be resolved as single-molecule events [17, 29–31]. Recently we have applied this idea to probe the mechanisms of antibiotic transport across bacterial walls [32].

Figure 4 shows that addition of a β-lactam antibiotic, ampicillin, introduces excess noise whose power spectra can be described by single Lorentzians. These spectra can be used to quantify the thermodynamics and kinetics of drug translocation [26–28, 32–34].

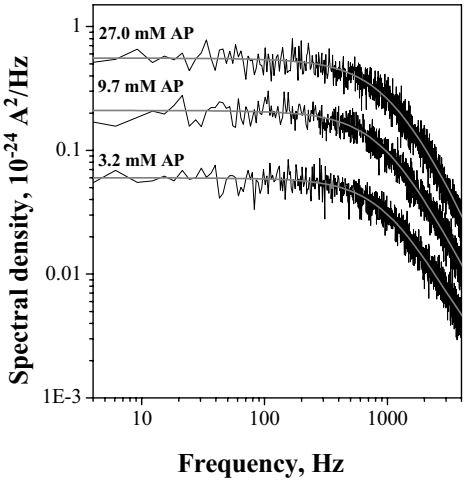

Fig. 4. Addition of the antibiotic ampicillin generates an excess conductance noise that can be used to characterize drug translocation through OmpF channels [32]. Power spectral densities of current fluctuations at three different ampicillin concentrations demonstrate that the process is well described by a simple two-state Markovian process. Solid lines through the spectra are drawn as Lorentzians with the relaxation time of $1.41 \cdot 10^{-4}$ s. The applied voltage was –100 mV, pH 6.0; other parameters as in Fig. 2.

Moreover, the characteristic time-scale of the process inferred from noise analysis suggests that the underlying single-molecule events can be time resolved. Figure 5 illustrates these time-resolved events.

Using this approach, we find that ampicillin and several other efficient penicillins and cephalosporins strongly interact with the residues of the constriction zone of OmpF channel. We conclude that, in analogy to the substrate-specific channels that evolved to bind certain metabolite molecules, antibiotics have 'evolved' to be channel-specific.

Molecular modeling [32] suggests that the charge distribution on the ampicillin molecule complements the charge distribution at the narrowest part of the bacterial porin. The interaction of these charges creates a region of attraction inside the channel that compensates for the entropy loss and facilitates drug translocation through the constriction zone. Therefore, the molecular mechanism that accounts for higher permeability rates of certain drugs can be traced down to their charge patterns as well as their 3D structure.

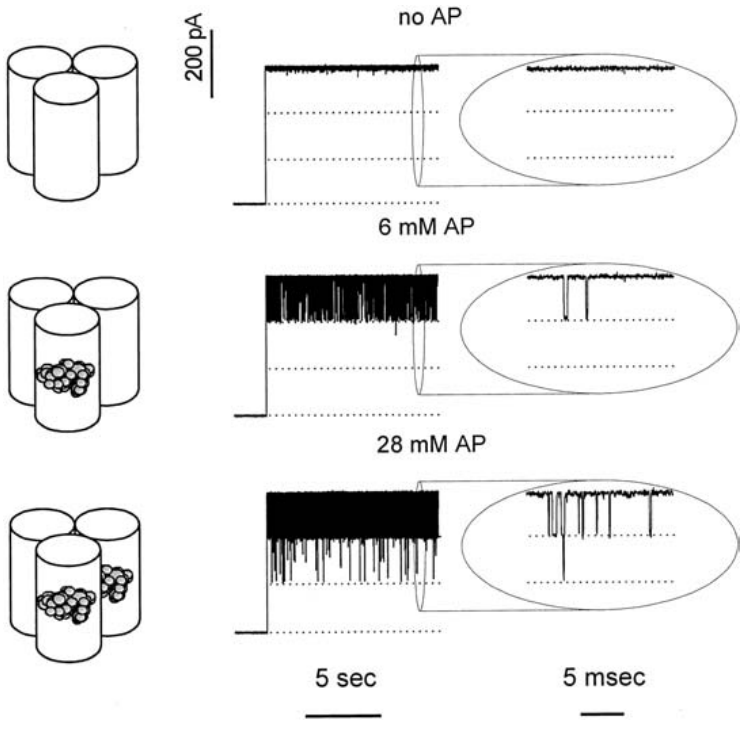

Fig. 5. Penetrating ampicillin (AP) molecules modulate ion current through a single *OmpF* channel [32]. Membrane bathing solution was 1M KCl, pH 6.0, the applied voltage was -100 mV. Top: In the absence of antibiotic the ion movement is mainly determined by the geometry and the surface properties of the channel pore. The ion current is stable, no interruptions are seen even at the high resolution recording shown on the right. Middle: In the presence of ampicillin in small concentrations one of the three *OmpF* pores gets spontaneously blocked by a translocating drug molecule. At the time resolution of 0.15 ms (left) blockage events look like downward spikes. At the higher resolution of 0.015 ms (right) they are seen as well-defined steps to 2/3 of the open channel current and back. Time between blockage events and their width provide both thermodynamic and kinetic parameters of antibiotic-pore interactions. Bottom: At higher ampicillin concentrations channel blockades are more frequent. Sometimes they overlap in time, leading to a transient reduction of ion current to 1/3 of its initial value.

5. Conclusions

Noise and fluctuation analysis of single protein nanocontacts provides important information about their structure and function. It advances our understanding of protein enzymatic activity by allowing us to study protein dynamics and interactions with different solutes at the single molecule level. Essential practical consequences include deeper understanding of molecular mechanisms of human illnesses and disorders, and further insights into the efficacy of newly designed drugs.

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